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Please find below and/or attached an Office communication concerning this application or proceeding.

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Office Action Summary	Application No. 08/866,279	Applicant(s) Dymecki
	Examiner Anne-Marie Baker, Ph.D.	Group Art Unit 1632

☐ Responsive to communication(s) filed on _____.

☐ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) 1-49 is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) 1-49 is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 3

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

Claims 1-49 are pending in the instant application.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 4 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for integration of Flp-recognition sequences into the genome of a mouse, does not reasonably provide enablement for introducing the Flp-recognition sequences in such a way as to generate a mosaic transgenic mouse wherein at least two diploid cells have different number of Flp-recognition sequences. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The specification does not provide an enabling disclosure for how to make and use transgenic mice that are mosaic with respect to the integrated Flp-recognition sequences. The specification does not provide any guidance for making a transgenic mouse wherein the cells have different numbers of Flp-recognition sequences integrated into the genome. The specification also does not provide any guidance on how one would use such animals.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 15, 41, 42, and 47 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 15 and 47 are indefinite in the recitation of the transgene or nucleic acid selected from the group consisting of a developmental gene or an essential gene. It is unclear what is meant by the term "essential gene" because it is not evident in what respect the gene is considered to be essential. The gene could be essential for the viability of the organism or essential for a particular function. Additionally, it is unclear what is meant by a "developmental gene" because such a gene could function in the control of development or could be differentially expressed in different stages of development while not playing a role in the control of development.

Claims 41 and 42 are indefinite in the recitation of a "developmental gene" for the reasons described above.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 4-19, 22-27, 29-36, 41-43, 45, and 48 are rejected under 35 U.S.C. 102(b) as being anticipated by Kilby et al., 1993.

The claims are directed to transgenic mice comprising an Flp transgene and one or more FRT sequences (Flp recognition sequences), wherein the Flp transgene is expressed at a level of recombinase activity sufficient to catalyze recombination between Flp recognition sequences of the cell. The claims are also drawn to a method of *in vivo* genetic engineering wherein a transgenic mouse comprising an Flp transgene and at least two Flp-recognition sequences, expresses the Flp transgene and catalyzes recombination between the two Flp-recognition sequences of the cell.

Kilby et al. teach that site-specific recombinases can be used in transgenic mice to produce an *in vivo* system in which further DNA manipulations can be achieved by selective expression of the recombinase. The reference teaches that the recombinases cre, FLP and R all belong to the λ integrase family of recombinases and show striking similarities in the types of reactions they carry out, the structure of their target sites, and the mechanism of recombination (p. 413, paragraph 3). The orientation of the target sites determines what type of reaction takes place. Recombination between sites in a direct (head-to-tail) repeat causes excision of the intervening DNA as a circular molecule. The reverse of deletion is the targeted integration of circular DNA into the linear molecule; this has potential in gene targeting to a specified chromosomal locus. Recombination between two target sites in an inverted (head-to-head) orientation on the same molecule will invert the DNA between them (page 413, paragraph 5). This mechanism can be exploited to activate or inactivate genes selectively upon induced expression of the recombinase. Both the Cre-lox system and the FLP-FRT system have been used extensively *in vitro* to study gene expression and mechanisms of recombination, as well as to devise strategies for targeted integration of exogenous DNA into the genomic DNA of the cell. The cre-lox system has also been used in transgenic mice and has demonstrated the utility of the system for activation of a transgene bearing lox target sites (see e.g., p. 417, paragraph 2). The FLP-FRT system has been used in embryonic stem cells to delete an FRT-flanked selectable marker. The FLP-

FRT system has also been used *in vivo*, in *Drosophila*. Flies transgenic for a heat shock promoter-FLP construct were crossed to flies whose sole copy of the cell-autonomous eye-color gene *white* was flanked by directly repeated FRT sequences. Deletion of the *white* gene, mediated by FLP expression in both somatic cells and germ line cells, caused white patches in the eye. The extent of mosaicism correlated with the severity and duration of the heat shock. By varying the timing of the heat shock, eye development could be visualized proceeding from the posterior to anterior (p. 418, paragraph 3). Thus, *in vivo* developmental studies have successfully employed the FLP-FRT system. Kilby et al. noted that site-specific recombinases would be useful in transgenic animals for applications in developmental biology, in activating or removing genes at particular stages. Furthermore, they stated that such controlled gene expression could mark a clone of cells for lineage studies, or allow the effect of lethal or deleterious sequences to be studied in a particular cell type or developmental stage (p. 417, paragraph 2). It is noted that although Kilby et al. did not reduce to practice the generation and use of transgenic mice with the FLP recombinase gene and FRT target sequences they provided all of the teachings necessary to enable one skilled in the art to make and use the transgenic mice claimed in the instant invention, including the motivation to use such animals for developmental studies, cell lineage studies, and controlled gene activation/inactivation studies in conjunction with cell-type specific gene expression.

Claims 1, 2, 4-13, 22-27, 29-33, 41-43, 45, and 48 are rejected under 35 U.S.C. 102(b) as being anticipated by Wigley et al., 1994.

The claims are directed to transgenic mice comprising an Flp transgene and one or more FRT sequences (Flp recognition sequences), wherein the Flp transgene is expressed at a level of recombinase activity sufficient to catalyze recombination between Flp recognition sequences of the cell. The claims are

also drawn to a method of *in vivo* genetic engineering wherein a transgenic mouse comprising an Flp transgene and at least two Flp-recognition sequences, expresses the Flp transgene and catalyzes recombination between the two Flp-recognition sequences of the cell.

Wigley et al. disclose an approach for accomplishing site-specific transgene insertion using the FLP recombinase system. Their approach seeks to allow single copy insertion of transgenes into a defined site in animal genomes (p. 585, paragraph 4 of Introduction). The method of transgenesis is outlined in the following steps: (1) gene targeting to introduce an FRT site into a specific genomic locus in ES cells, thereby creating the 'transgene acceptor site'; (2) single-copy insertion of transgenes into the targeted FRT site using FLP recombinase; and (3) generation of transgenic animals from the modified ES cells (p. 586, paragraph 6). Wigley et al. contemplate using the FLP recombinase system to excise marker genes from their transfected ES cells. ES cells with an FRT-neo-FRT cassette integrated into the histone H4 gene locus will be transfected with a source of FLP recombinase in order to excise the *neo* gene, thereby generating a single FRT site in the H4 locus, i.e. the transgene acceptor site (TAS) (p. 586, column 2, paragraph 6). FLP recombinase will be used to insert genes, in a single copy, into the TAS. This will involve transfection of the TAS ES cell line with a source of FLP plus a plasmid containing the gene of interest (with regulatory elements) linked to a single FRT. The modified ES cells will then be used to generate transgenic animals (p. 586, column 2, paragraph 7). Wigley et al. indicate that a pulse of FLP activity can be supplied to ES cells by transfecting the FLP gene under the control of the interferon-inducible human '6-16' promoter and that this method has been used successfully to demonstrate FLP-mediated excision (p. 587, paragraphs 4-7). It is noted that although Wigley et al. did not reduce to practice the generation and use of transgenic mice with the FLP recombinase gene and FRT target sequences they provided all of the teachings necessary to enable one

skilled in the art to make and use the transgenic mice claimed in the instant invention, including the motivation to use FLP-mediated recombination to excise marker genes, such as the neomycin resistance gene.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 2, 4-13, 15, 22-27, 29-33, 37-43, 45, 47, and 48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lakso et al., 1992, Wigley et al., 1994, Marx, 1993, Marshall, 1989, and Bieche et al., 1992.

The claims are drawn to a method of *in vivo* genetic engineering using FLP-FRT transgenic mice wherein recombination causes activation of an oncogene or inactivation of a tumor suppressor gene in the cell, thereby transforming the cell and establishing a probability of developing cancer in the transgenic mouse.

Lakso et al. generated transgenic mice carrying the murine lens-specific αA-crystallin promoter and the simian virus 40 large tumor-antigen gene sequence, separated by a 1.3-kilobase-pair Stop sequence that contains elements preventing expression of the large tumor-antigen gene and Cre recombinase recognition sites. These transgenic mice were mated with transgenic mice expressing the Cre recombinase under control

of the αA-crystallin promoter or the human cytomegalovirus promoter. All double-transgenic offspring developed lens tumors. Analysis confirmed that tumor formation resulted from large tumor-antigen activation via site-specific, Cre-mediated deletion of Stop sequences. Thus the reference teaches the concept of using a recombinase system to activate an oncogene in a specific cell type. Although Lakso et al. do not teach the use of the FLP recombinase system in transgenic mice, they do suggest that the FLP recombinase will be useful in directing precise site-specific DNA rearrangements in transgenic animals, and emphasize that the FLP recombinase of *Saccharomyces cerevisiae* has been shown to be proficient for recombination in both *Drosophila* and in cultured mammalian cells (p. 6235, paragraph 2)

Wigley et al. disclose the potential use of the FLP recombinase system in transgenic mice, as described above. Wigley et al. do not teach the oncogenes disclosed in Claim 39 nor the tumor supressor genes disclosed in Claim 40.

Marshall reviews a number of known oncogenes, including ABL, BCL1, BCL2, ERBA, ERBB, ERBB2, ETS1, FGR, FOS, JUN, MYB, MYC, NRAS, PIM1, SRC, and YES and discusses the mechanisms of oncogene activation. Marshall does not teach the tumor supressor genes contemplated for use in the FLP recombinase-transgenic mice.

Marx discloses a number of known tumor suppressor genes, including APC, DCC, NF1, NF2, RB1, and WT1. As pointed out by Marx, it is well-established in the art that alterations that inactivate tumor suppressor genes play a key role in the development of human tumors. Marx does not discuss the tumor suppressor genes BRCA1, BRCA2, or TP53.

Bieche et al. review the role of the known tumor suppressor genes BRCA1, BRCA2, and TP53 and their mutations in development of breast cancer.

Since the Cre-lox system is analogous to the FLP-FRT recombinase system, and since it would have been desirable to use the eukaryotic FLP-FRT system in mammalian cells in place of the bacteriophage P1 Cre recombinase system, one would have been motivated to substitute the FLP-FRT system for the Cre-lox system, using it in a similar manner to activate oncogenes that have been integrated into the genome of a mouse as specially designed transgene constructs. Since it is well-known in the art that activation of oncogenes and inactivation of tumor suppressor genes both play a major role in neoplastic transformation, one would have been motivated to use the FLP recombinase system for both the activation of oncogenes as well as for the inactivation of tumor suppressor genes in order to study malignant transformation in a particular cell type. One would have anticipated a reasonable expectation of success because the Cre-lox system had already been successfully employed to activate an oncogene in a transgenic mouse. Given the fact that the FLP recombinase system is analogous to the Cre recombinase system and functions in a manner that is mechanistically identical to the activity of Cre, and given that the oncogenes and tumor suppressor genes contemplated for use in the method of Claim 37 are all known in the art, it would have been obvious to one of skill in the art at the time of the invention to have used the FLP recombinase system in a transgenic mouse carrying an appropriate transgene construct comprising FRT sequences such that an oncogene could be activated or a tumor suppressor gene could be inactivated upon expression of the recombinase.

One would have been motivated to have combined the teachings of Lakso et al., Wigley et al., Marshall, Marx, and Bieche et al. in order to generate a transgenic mouse useful for the study of neoplastic transformation, *in vivo*.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 3, 21, 28, 44, 46, and 49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wigley et al., 1994, Panigrahi et al., 1992, O'Gorman et al., 1991, Wahl et al. (US Pat. No. 5,654,182), 1997, Hartley et al., 1980 and Buchholz et al., 1996.

The claims are drawn to a transgenic mouse comprising the Flp transgene and the Flp-recognition sequence, wherein the Flp-recognition sequence is SEQ ID NO:14 or SEQ ID NO:15 and the Flp transgene encodes the amino acid sequence SEQ ID NO:17 or SEQ ID NO:19, and a method of using such a transgenic mouse for *in vivo* genetic engineering.

Wigley et al. disclose the potential use of the FLP recombinase system in transgenic mice as described above under the 102(b) rejection. Wigley et al. do not disclose the amino acid sequence of the FLP recombinase or the DNA sequence of the FRT target sequence.

Panigrahi et al. disclose the DNA sequence of the FRT target sequence referred to as SEQ ID NO:15 in the instant application (see Figure 1).

O'Gorman et al. disclose the DNA sequence of the FRT target sequence referred to as SEQ ID NO: 14 in the instant application (see Reference Note 2 and p. 1351, column 3, paragraph 2).

Either Wahl et al., 1997 (US Pat. No. 5,654,182) or Hartley et al., 1980 disclose the amino acid sequence of FLP recombinase referred to as SEQ ID NO:19. See SEQ ID NO:1 and SEQ ID NO:2 of US Pat. No. 5,654,182 or Figure 2 of Hartley et al.

Buchholz et al. disclose a temperature-sensitive mutant of FLP recombinase wherein the phenylalanine at position 70 is mutated to leucine. This amino acid change, in conjunction with the complete sequence disclosed by Hartley et al., as more concisely depicted by Wahl et al. (US Pat. No. 5,654,182), corresponds to SEQ ID NO:17.

Since the FLP recombinase system is desirable for use in a transgenic mouse for *in vivo* manipulation of the integrated transgene constructs, one would have been motivated to construct such a transgenic mouse using the amino acid sequence of FLP recombinase and the DNA sequences of the FRT target site, from the information available in the prior art. One would have anticipated a reasonable expectation of success because the FLP recombinase gene and FRT target sequences had already been used successfully in cultured mammalian cells as well as in transgenic *Drosophila* (as described above in the discussion of the Kilby et al. reference). Therefore, it would have been obvious to one of skill in the art at the time of the invention to have used the sequence information available in the literature to construct transgenes for the generation of transgenic mice carrying a functional FLP recombinase gene and FRT target sequences.

One would have been motivated to have combined the teachings of Wigley et al., 1994, Panigrahi et al., 1992, O'Gorman et al., 1991, Wahl et al. (US Pat. No. 5,654,182), 1997, Hartley et al., 1980 and Buchholz et al., 1996 in order to generate a transgenic mouse useful for *in vivo* genetic manipulation.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 1, 12, 15, 20, 24, 43, and 47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Orban et al., 1992 and Wigley et al., 1994.

The claims are drawn to an FLP-FRT transgenic mouse wherein the transgene flanked by the FRT target sequences is selected from the group consisting of developmental gene, essential gene, cytokine gene, neurotransmitter gene, neurotransmitter receptor gene, oncogene, tumor suppressor gene, selectable marker, and histochemical marker. Furthermore, the recombinase activity is regulated by a factor selected from the group consisting of chemical, developmental stage, temperature, and tissue type. Claims 24 and 43 are drawn

to a method of *in vivo* genetic engineering wherein Flp-mediated recombination identifies a cell lineage in the transgenic mouse.

Orban et al. disclose the use of the Cre-lox system in transgenic mice wherein the the recombination target transgene is a β-galactosidase gene flanked by *loxP* target sequences. Mice carrying the Cre recombinase transgene under the control of the thymocyte specific *lck* promoter were bred to mice carrying the *loxP*-β-gal-*loxP* transgene construct also under control of the *lck* promoter to obtain doubly transgenic mice. These mice exhibited tissue-specific DNA recombination as a result of Cre expression (p. 6862, paragraph 3 and Figure 2). Southern analysis indicated that the recombination occurred specifically in thymocytes of the doubly transgenic mice and not in tail DNA. The recombined DNA structure also was not present in parental thymocyte DNA from either the Cre or *loxP*-β-gal-*loxP* transgenic mice. Orban et al. also disclose that Cre-mediated recombination provides a heritable marker for mitoses following the loss of Cre expression (Abstract). Although Cre expression was not detected in the splenic T cell subpopulation, a high degree of recombination within the *loxP*-β-gal-*loxP* transgene array was detected, implying that these T cells derived from thymocytes in which recombination had occurred. Thus, the heritable nature of Cre-mediated recombination provides a methodology for cell fate determinations in mammals. This method depends on tissue-limited expression of the recombinase transgene, wherein thymocytes that express Cre recombinase develop into mature T cells that are not capable of expressing Cre recombinase. Although Orban et al. do not teach the use of the FLP recombinase system in transgenic mice, they do emphasize that Cre activity appears mechanistically identical to that of yeast FLP recombinase and that both recombinases have been used in cultured eukaryotic cells and *in vivo* in Drosophila to direct site-specific recombination (p. 6861, paragraph 2). Thus, Orban et al. teach the use of a recombinase system analogous to the FLP system in transgenic mice wherein the transgene flanked by the target sequences encodes a histochemical marker as claimed in the

present invention for the FLP system. Furthermore, the recombinase activity is regulated by tissue type due to the use of the *lck* promoter which exhibits thymocyte-specific activity. As in the instantly claimed invention, the recombination event can be used to identify a cell lineage in the transgenic mouse.

Wigley et al. disclose the potential use of the FLP recombinase system in transgenic mice, as described above.

Since the Cre-loxP system is analogous to the FLP-FRT recombinase system, and since tissue-specific expression of genes is a method known in the art to be extremely useful for analysis of gene expression and gene function, one would have been motivated to use regulated expression of marker genes, either histochemical markers or selectable markers, in the context of the FLP-FRT recombinase system in order to achieve high-efficiency recombination in a eukaryotic system with the combined advantage of controlled expression of easily detectable genes and gene products. One would have anticipated a reasonable expectation of success because the analogous Cre-loxP system had already been successfully employed to analyze recombination events and cell lineage in transgenic mice using a histochemical marker gene in a tissue-restricted manner. Therefore, it would have been obvious to one of skill in the art at the time of the invention to have used the FLP recombinase system in a transgenic mouse carrying a marker gene, wherein either or both the marker gene and/or the recombinase gene are under the control of a tissue-specific control element (such as a promoter or locus control region).

One would have been motivated to have combined the teachings of Orban et al. and Wigley et al. in order to develop an *in vivo* system useful for the analysis of cell fate or regulated gene expression, wherein genes can be selectively turned on or off at will, depending on the type of regulatory control region included in the transgene constructs.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

No claim is allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anne-Marie Baker whose telephone number is (703) 306-9155. The examiner can normally be reached Monday through Friday from 8:00 AM to 5:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jasemine Chambers, can be reached on (703) 308-2035. The fax phone number for the organization where this application or proceeding is assigned is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Anne-Marie Baker, Ph.D.
September 14, 1998

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